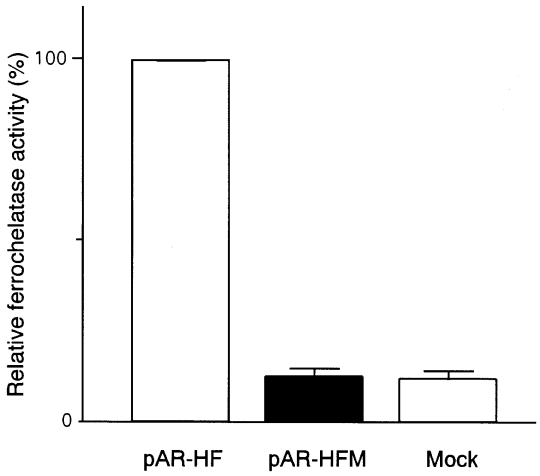

Figure 3. Demonstration of a functional mutation with bacterial expression assay. Mutated FECH protein expressed in *E.* coli BL21 has no residual activity. Data are shown as the percentage of the FECH activity with normal control, based on the mean ± SD of triplicate experiments. The average activity of cell extracts from pAR-HF transformed bacteria was 10.2 nmol zinc-mesoporphyrin formed per min at 37°C.

transformed bacteria was similar to that of a mock cDNA-transformed one, indicating that the mutated FECH did not exhibit any activity (**Fig 3**).

The location of mutations in the FECH gene is highly heterogeneous and there are no mutation hot spots. The predominant type of FECH gene mutations are single-nucleotide substitutions in the coding region (42%), followed by exon skipping (34%) and small deletions and insertions (21%) (Rufenacht *et al*, 1998). This is the first instance of an amino acid insertion in the FECH protein. The transition of A to G at 4 bases from the 3′ terminus of intron 4 (IVS4^{-4}, a→g) leads to in-frame insertion of three bases in ferrochelatase mRNA (**Fig 2*B***). It is suggested that recognition of the 3′ splice site a(–2)g(–1) by the splicing factor U2AF^{35} (Wu *et al*, 1999) was disturbed due to the mutation at a(–5)g(–4). As a result of misreading the 3′ acceptor splice site, aberrant splicing would occur. Aberrant splicing has been classified into four types: exon skipping, cryptic site activation, intron retention, and new site creation (Nakai and Sakamoto, 1994). Our case corresponds to the last type. The observed frequency of point mutations at position –4 within 3′ splice sites was 0 among 101 different point mutations in the vicinity of mRNA splice junctions (Krawczak *et al*, 1992). To our knowledge, a mutation at position –4 that generated a novel acceptor splice site in FECH gene is rare and critical for this patient.

It is not too surprising that his mother was asymptomatic, though she was found to be heterozygous for the mutation. The reason is that EPP is thought to be inherited with low clinical penetrance and may be considered as an inherited disorder that does not strictly follow recessive or dominant rules (Gouya *et al*, 1996). Other factors such as the expression level of the wild-type allele and environmental effects may contribute to modulation of the phenotype.

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Mechanical Stress Induced by Hyperosmolarity

To the Editor:

We have recently read the article by Dascalu *et al* (2000) in which our work was cited and hereby wish to comment on what we believe to be inaccuracies in the interpretation of our cited article.

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Reprint requests to: Dr. Stefan Kippenberger, Department of Dermatology and Venerology, Klinikum der Johann Wolfgang Goethe Universität, Theodor-Stern-Kai 7, D-60590 Frankfurt/Main, Germany.

Several lines of evidence have supported the assumption that mechanical forces applied to human cells are able to modulate fundamental events like proliferation and differentiation (Chen *et al*, 1997). Our group has already shown that different qualities of mechanical stimuli provoke different cell responses (Görmar *et al*, 1990; Kippenberger *et al*, 1999, 2000a, b). The application of cell stretch to human keratinocytes *in vitro* amplifies the proliferation cascade, whereas mechanical pressure was shown to increase differentiation signals. In the October 2000 issue of the *Journal of Investigative Dermatology*, Dascalu *et al* subjected human keratinocytes to hyperosmotic stress and discussed the cellular responses as a

quality of mechanical stress. In particular, they observed a growth inhibition due to hyperosmotic stress and referred to data from our group (Görmar *et al*, 1990) and that of Takei *et al* (1997), which in their opinion, were contradictory. This is not the case, as both cited publications made use of different qualities of mechanical stress. In the work of Takei *et al* mechanical stretch lead to enhanced proliferation of human keratinocytes, whereas our work made use of mechanical pressure giving an increase in differentiation parameters. Both these findings show no conflict and are well in harmony with the *in vivo* situation where skin stretch leads to skin enlargement without changes in skin thickness. Examples for this functional connection are given by abdominal skin growth during pregnancy or the use of skin expanders for cosmetical surgery. On the other hand, the application of pressure to the skin organ repels proliferation and instead triggers differentiation processes (hyperkeratosis, acanthosis).

Dascalu *et al* applied hyperosmotic stress to human keratinocytes and interpreted this stimulus as being mechanically relevant without showing it. They claim that hyperosmotic conditions represent a form of mechanical pressure. This is an error in logic as hyperosmotic conditions initially lead to cell shrinkage and therefore to a decrease in intracellular pressure accompanied by reduced turgidity. In contrast, the application of mechanical pressure leads to cell compression that might also increase intracellular pressure. In conclusion, we strongly suggest investigating how hyperosmotic stress alters the cell volume in the system presented and how compensating mechanisms like regulatory volume increase (RVI) counteract osmotic shrinkage. At present, the reported data give no evidence that hyperosmotic stress represents a form of mechano-stress. In this context it is essential to use more than just one osmotically active substance in order to delineate measured cell responses to the presence of hyperosmolarity. Therefore, it maybe worthwhile to also make use of membrane-permeable osmotic active substances like urea that would be able to substantiate the relevance of cell volume in this context.

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Reply

To the Editor:

In reply to the remarks of Kippenberger *et al*, we would kindly refer the letter's authors to the ample work performed by Parsegian and coworkers, which demonstrates in detail that osmotic stress causes structural changes of macromolecules via crowding and hydration effects (Rand, 2000). For example, previous attempts to change the volume of ion channels by hydrostatic pressure were replaced by the elegant method of osmotic stress based on maintaining a difference in solute concentrations between the bathing solution and the water-filled ion channels, leading to decreased channel volume. Therefore, both mechanical pressure and osmotic stress cell membrane affect intracellular structures.

As to the other points, the periodical mechanical stimulation, employed by Gormar *et al* (1990), is the equivalent to a cyclic strain used by Takei *et al* (1997) and resulted in proliferative changes as reported. On the other hand, the technique of constant stretch gives different results as described by Kippenberger *et al*.

The presentation of skin stretch as a cause of "skin enlargement without changes in skin thickness" is an oversimplification of the clinical and pathologic skin conditions that were cited by Takei *et al* (1997). For example, the dermis might become thinner, and a consideration of the length of time of the mechanical stimulus application should be taken into account. Furthermore, it is difficult to draw conclusions using keratinocytes as the sole cellular model, without further evaluation of a fibroblast-keratinocyte 3D model, with a specific hormonal milieu relevant to the clinical comparison.

Cells respond to different mechanical stimuli by membrane deformation, to be followed by activation of specific signal transducers. Specifically, it should be noted that a hyperosmotic stress leads cell shrinkage, causing a fast increase in the ratio of cell surface to volume. The cellular deflation over a rigid skeleton leads to both membrane stretching and folding of the plasma membrane in various areas, thus mimicking mechanical stress. Additionally, the intracellular pressure mentioned by Kippenberger *et al* is not known as a major sensor in keratinocytes, and we believe the proliferative and differentiation effects are related to ion-channel activation and to interplay of calcium homeostasis and gradient, which are critical to the epidermis.

In conclusion, we delineated mechanical pathways of activation and their end-point results of proliferation-differentiation balance. The general study of volume control in keratinocytes, whose physiologic relevance is not clear to us, was not within the scope of our study.

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